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Comparative Evaluation of a Modified Zinc Sulfate Flotation Technique

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A modified zinc sulfate flotation technique using Formalinized fecal specimens (F-$ZnSO_4$) was compared to the Formalin-ether (FE) concentration method for the laboratory diagnosis of intestinal parasites. Many laboratories have difficulty storing, using, and disposing of ether and need a procedure for concentrating fecal specimens which does not require ether. Comparative data were obtained for the recovery of protozoan cysts and helminth eggs and larvae from feces preserved in Formalin less than and longer than 1 month. Whereas the FE method was found generally to be more efficient, F-$ZnSO_4$ was apparently more effective for the recovery of some species of parasites. F-$ZnSO_4$ was not satisfactory for recovery of schistosome eggs. We conclude that, except for schistosomes, F-$ZnSO_4$ compares favorably to the FE method for detecting infections of clinical significance.

The original zinc sulfate centrifugal flotation ($ZnSO_4$) technique (2) since its development has been variously modified for use as a routine concentration procedure for detecting intestinal helminths and protozoa (4). In studies comparing the recovery of parasites by the original $ZnSO_4$ method, and some modifications, with recovery by the Formalin-ether sedimentation (FE) technique, the latter generally has appeared more efficient (4-7). However, the FE method has the disadvantage of employing ether, which is combustible and requires special safety precautions for storage, use, and disposal.

One modification of the $ZnSO_4$ method is the Formalin-zinc sulfate flotation (F-$ZnSO_4$) technique (3, 4), which incorporates some of the advantages of the FE technique and has been employed routinely in the Indiana University laboratory for 6 years and in the Indiana State Board of Health for 15 years. This method eliminates washing steps and utilizes Formalin fixation to clear internal structures of protozoan cysts and prevent distortion commonly associated with salt solutions of high specific gravity. Furthermore, the method is adaptable to variations in individual laboratory schedules in that the preliminary fixation of fecal specimens can be performed separately from the actual concentration for microscopic examination.

Our experience with this particular procedure has suggested that it is an acceptable substitute for the FE method. The present investigation was undertaken to compare the F-$ZnSO_4$ and FE techniques, since no such study has previously been published and because many laboratories desire a concentration technique that does not present the storage and disposal problems found with ether. The study also compares the recovery of parasites from fecal specimens fixed in Formalin less than 1 month and longer than 1 month.

MATERIALS AND METHODS

Specimens. Fecal specimens known to contain one or more species of protozoa and helminths were obtained from laboratories in the United States, Puerto Rico, Japan, and Egypt. Specimens were submitted either in screw-cap mixing bottles (33 by 70 mm) containing 15 ml of 10% Formalin and five glass beads (5 mm), or in vials of Formalin only and subsequently transferred to the mixing bottles for processing. Approximately 1 part of feces to 3 to 5 parts of 10% Formalin was employed. The date of preservation in Formalin was requested for each sample.

Specimens were separated into two groups according to the length of time they had been preserved in Formalin: less than 1 month, and longer than 1 month. Samples were given identification numbers and thereafter processed in a random manner as blind specimens. Prior to concentration, they were mixed for 10 to 15 s on a Vortex mixer set at maximum speed. Equal portions of each specimen were concentrated by both methods, and these samples of finely suspended feces were always obtained immediately after mixing. Each specimen was concentrated by both methods within 4 days.

F-$ZnSO_4$ method. For the F-$ZnSO_4$ flotation modification (3, 4), each sample of fecal-Formalin suspension was strained through one layer of gauze into a conical paper cup and immediately poured into a round-bottom tube (100 by 16 mm) to within 3/4 inch (ca. 19.05 mm) of the rim. The suspension was then centrifuged for 3.5 min at 1,800 rpm (750 × *g*). (All centrifugations were effected without mechanical braking.) The supernatant was decanted, the last drop was drained onto a clean section of paper towel, and aqueous $ZnSO_4$ solution (1.195 to 1.200 specific gravity) was added to within 1 inch (ca. 25.4 mm) of the rim of the tube. The packed sediment was then resuspended, using two applicator sticks, until no coarse particles remained. This suspension was immediately centrifuged at 1,500 rpm (500 × *g*) for 1.5 min, transferred without agitation to a rack which held it upright, and allowed to stand for 1 min to compensate for any disruptive movement occurring during transfer from the centrifuge. With a wire loop 7 mm in diameter, bent at a right angle to the stem, two loops of the surface film were transferred to a drop of 0.85% saline and to a drop of Dobell and O'Connor iodine (1, 4) on a glass slide (3 by 2 inches; ca. 76.2 by 50.8 mm) for wet-mount examination. The specific gravity of the zinc sulfate solution was checked frequently with a calibrated hydrometer for heavy liquids with a specific gravity range of 1.00 to 1.22 throughout the study.

FE method. The FE method as described for use with Formalin-fixed feces (4) was employed in this study. Briefly, 10 ml of strained fecal-Formalin suspension, as prepared for the F-$ZnSO_4$ modification, was transferred to a conical centrifuge tube, ether was added, and the tube was stoppered, shaken vigorously, and centrifuged. The top three layers were decanted, and a saline and an iodine wet mount were prepared from the sediment.

Examination of wet mounts. One saline and one iodine preparation were systematically examined from each concentrated FE sediment or F-$ZnSO_4$ surface film. Microscopy was performed by one of three individuals competent in identifying intestinal protozoa and helminths. The two concentration procedures were not performed concurrently, and if the same parasitologist examined mounts from both the FE and F-$ZnSO_4$ concentrations of a given specimen, it was by chance.

Findings were recorded as to species, parasitic stages, and semiquantitative numbers, with five or fewer organisms per cover slip mount designated as rare; 6 to 20, few; 20 to 40, moderate; and more than 40, many.

Determination of relative efficiencies of recovery. Since the objective of this study was to compare only the relative efficiency of the two concentration techniques in recovering the individual species of parasites, all data analyses were based on acceptance of 100% recovery of a species being equal to the total specimens in which it was found, regardless of the method or combination of methods yielding the recovery.

RESULTS

In the 262 Formalin-fixed fecal samples concentrated by both the F-$ZnSO_4$ and the FE methods for Formalinized specimens, a total of 505 intestinal parasites were found (263 as protozoan cysts, 224 as helminth eggs, 18 as helminth larvae).

Table 1 shows the relative efficiency of the two techniques for recovering stages of the individual species. Of the 505 findings, 379 were detected by both methods. The FE technique yielded 91% (462) of the total parasites found and F-$ZnSO_4$ yielded 84% (422). The greatest difference between the two methods was observed in the recovery of helminth eggs, with 89% of the total findings by either method being recovered by the FE method and 77% by F-$ZnSO_4$. When data pertaining to protozoa only were considered, this difference was less (FE, 94%; F-$ZnSO_4$, 89%).

The efficiency of each method for the recovery of individual species is shown in Table 1. When the null hypothesis was applied to determine significant differences (Table 1) in recovery by the two methods of the 19 species studied, significant differences were observed for four species: *Entamoeba coli, Endolimax nana,* hookworm, and *Schistosoma mansoni.*

The recovery of parasites from feces preserved in Formalin less than 1 month and longer than 1 month is shown in Table 2. Storage time appeared to have some effect on certain parasites. Using the basis described in Materials and Methods for determining relative efficiency, 100% recovery of *Entamoeba histolytica* cysts was obtained by both methods from specimens preserved in Formalin less than 1 month, whereas only 50% recovery was obtained by the FE concentration of feces preserved in Formalin longer than 1 month. The F-$ZnSO_4$ method apparently was more efficient for detecting *Entamoeba hartmanni* cysts and *Clonorchis sinensis* eggs in the older Formalin-preserved specimens, whereas the relative efficiency of the FE technique increased from 50 to 100% for *Enterobius vermicularis* eggs when only long-term preserved specimens were concerned. Variances of lesser degrees were observed for other species.

DISCUSSION

Although, in general, the FE method was the more sensitive of the two, variances related to the species of parasite and length of fixation time in Formalin were observed.

In comparing the recovery of organisms from feces preserved in Formalin less than and longer than 1 month, differences observed suggest that fecal specimens preserved in Formalin for extended periods of time may not always be suitable for evaluating concentration techniques for

TABLE 1. *Recovery of parasites by F-$ZnSO_4$ and FE concentration methods from 262 fecal specimens*

Parasites	Total identifications	F-$ZnSO_4$ total/only[a]	FE total/only[a]	Both methods	Probability[b]
Protozoa (cysts)	263	234	247	218	
Entamoeba histolytica	22	22/5	17/0	17	<0.1
Entamoeba hartmanni	34	29/3	31/5	26	>0.5
Entamoeba coli	71	61/2	69/10	59	<0.05+
Endolimax nana	68	58/2	66/10	56	<0.05+
Iodamoeba butschlii	17	14/2	15/3	12	>0.5
Giardia lamblia	46	45/2	44/1	43	>0.5
Chilomastix mesnili	5	5/0	5/0	5	
Helminths (eggs)	224	172	200	148	
Enterobius vermicularis	13	12/5	8/1	7	<0.5
Trichuris trichiura	56	39/8	48/17	31	<0.5
Ascaris lumbricoides	47	39/2	45/8	37	<0.5
Hookworm spp.	51	36/4	47/15	32	<0.02+
Taenia spp.	3	2/0	3/1	2	>0.5
Hymenolepis nana	13	13/2	11/0	11	<0.5
Hymenolepis diminuta	2	1/0	2/1	1	>0.5
Diphyllobothrium latum	11	11/0	11/0	11	
Schistosoma mansoni	10	2/0	10/8	2	<0.01+
Fasciola hepatica	1	1/0	1/0	1	
Clonorchis sinensis	17	16/3	14/1	13	>0.5
Helminths (larvae)	18	16	15	13	
Strongyloides stercoralis	13	13/2	11/0	11	>0.5
Hookworm spp.	5	3/1	4/2	2	<0.5

[a] Total recovered/recovered only by this method.
[b] Probability of significant difference of F-$ZnSO_4$ and FE results. +, Significant difference of <0.05.

use in laboratory diagnoses. In this study, the use of 1 month as the dividing time in attempting to obtain some indicaton of the effect of prolonged storage of feces in Formalin resulted in greater differences than had been expected.

One month had been selected as the separation time for the purposes of this study, since previous experience with the F-$ZnSO_4$ method by one of the authors had indicated that concentration results were comparable for specimens preserved in Formalin at intervals up to 1 month, but that at some time thereafter it appeared that reproducible results were not always possible. The effect of long-time storage is important because public health laboratories receiving Formalinized specimens through the mail may perform concentrations on specimens preserved longer than 1 month. In addition, the proficiency test specimens used for evaluation of laboratory performance are specimens held in Formalin for long periods of time.

Of the four species for which significant difference in total efficiency of the two methods was determined (Table 1), two, *E. coli* and *E. nana*, are considered harmless commensals and not clinically important. The greatest difference in percentage of recovery occurred with *E. nana* in the long-term preserved samples. The two species of protozoa capable of producing clinical disease, *E. histolytica* and *Giardia lamblia*, were detected in all but one instance by the F-$ZnSO_4$ technique, whereas five *E. histolytica* and two *G. lamblia* were missed by the FE method.

The other two species for which significant differences were found were hookworm and *S. mansoni*. The F-$ZnSO_4$ method in this sudy was found ineffective for the recovery of schistosome eggs.

Most specimens in this study were processed without prior knowledge of the species of parasites present. However, with specimens for recovering *S. mansoni* eggs, the situation was different in that we had to seek material containing specifically these eggs. Of the 21 special samples of fecal material with *S. mansoni* eggs, in only 9 instances were eggs found by either concentration method. These results suggest that neither method is fully adequate for detecting these eggs and that if schistosomiasis is suspected multiple specimens should be examined by one of the more sensitive methods recommended for the laboratory diagnosis of schistosomiasis.

Hookworm eggs, for which there was a signif-

TABLE 2. *Relative recoveries of parasites from fecal specimens according to preservation time in Formalin*

Parasites	<Month in Formalin			>Month in Formalin		
	Total identifications	F-$ZnSO_4$ (%)	FE (%)	Total identifications	F-$ZnSO_4$ (%)	FE (%)
Protozoa (cysts)						
Entamoeba histolytica	12	100	100	10	100	50
Entamoeba hartmanni	15	67	93	19	100	89
Entamoeba coli	45	84	96	26	88	100
Endolimax nana	34	91	100	34	79	94
Iodamoeba butschlii	13	85	92	4	75	75
Giardia lamblia	26	96	96	20	100	95
Chilomastix mesnili	4	100	100	1	100	100
Helminths (eggs)						
Enterobius vermicularis	10	90	50	3	100	100
Trichuris trichiura	26	73	92	30	67	80
Ascaris lumbricoides	29	86	93	18	78	100
Hookworm spp.	19	74	95	32	69	91
Taenia spp.	2	50	100	1	100	100
Hymenolepis nana	8	100	88	5	100	80
Hymenolepis diminuta	1	0	100	1	100	100
Diphyllobothrium latum				11	100	100
Schistosoma mansoni	7	0	100	3	67	100
Fasciola hepatica				1	100	100
Clonorchis sinensis	7	86	100	10	100	70
Helminths (larvae)						
Strongyloides stercoralis	8	100	88	5	100	80
Hookworm spp.				5	60	80

icant difference in recoveries, were found 51 times. Only 63% were detected by both methods. The F-$ZnSO_4$ method yielded 71% of the total 51 and FE, 92%. On further analysis of the data, in 24 of the 51 recoveries hookworm eggs were found in rare numbers (five or fewer eggs per cover slip). When these 24 specimens were excluded, there appeared to be no significant difference. Since hookworm egg counts of less than 2,600 per ml of feces are generally not considered indicative of clinically significant infections (4), it would seem that the finding or not finding of rare eggs by the F-$ZnSO_4$ concentration would also have little clinical significance. The effect of employing $ZnSO_4$ solution in a range of specific gravity measurements from 1.195 to 1.200 rather than precisely 1.200 may have had some small influence, and preservation of specimens in Formalin longer than 1 week may also have affected the flotation of hookworm eggs. Further investigation of the influence of these variables is proposed.

Although the FE concentration method was found to detect rare helminth eggs more frequently than F-$ZnSO_4$, in most instances of clinically significant infection there will be sufficient numbers of eggs to be detected with either method. In this study, 33% of the total helminth egg findings yielded egg counts of five or less.

The $ZnSO_4$ method of concentrating feces has generally been considered unsatisfactory for the recovery of operculated eggs. In this study, operculated eggs were found 29 times, 28 by F-$ZnSO_4$ and 26 by FE. Experience with F-$ZnSO_4$ modification in this study and in previously unpublished comparisons of the two methods at the Indiana University Medical Center and Indiana State Board of Health indicate that the F-$ZnSO_4$ technique is as efficient as the FE in the recovery of operculated eggs.

The F-$ZnSO_4$ modification has certain advantages. Wet mounts prepared from surface films have less background fecal detritus than do FE wet mounts; this is helpful for individuals with limited experience in microscopic screening. Protozoan cysts are cleared so that identification does not require the routine use of iodine, which can obscure important identifying structures. Neither cysts nor eggs are distorted by the high salt concentration when specimens have been initially fixed in Formalin. Schistosome eggs, when found, show no distortion and are easily identified. Zinc sulfate flotation techniques do not impose the hazards involving the use of ether. When the F-$ZnSO_4$ modification is employed, parasitological examinations can be readily coordinated with clinical laboratory schedules without reducing the quality of service.

The results of this comparison of the F-$ZnSO_4$

and Fe concentration techniques suggest that F-$ZnSO_4$ is an adequate method for the detection of most intestinal parasite species and is essentially comparable to the FE method when only clinically significant infections are considered.

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